510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k041043

B. Purpose for Submission:
New device

C. Analyte:
Varelisa PR3 ANCA EIA

D. Type of Test:
Semi-quantitative and qualitative (non competitive) enzyme immunoassay

E. Applicant:
Sweden Diagnostics (Germany) GmbH (Pharmacia Deutschland GmbH).

F. Proprietary and Established Names:
Varelisa® PR3 ANCA EIA

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.5660, Antinuclear Antibody Immunological Test System
2. Classification:
   Class II
3. Product Code:
   MOB, Antineutrophil cytoplasmic antibodies
4. Panel:
   Immunology 82

H. Intended Use:

1. Intended use(s):
   The Varelisa PR3 ANCA EIA kit is designed for the semi-quantitative and qualitative determination of proteinase 3 anti-neutrophil cyrtoplasmic antibodies (PR3 ANCA) in human serum or plasma to aid in the diagnosis of Wegener's granulomatosis.
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2. Indication(s) for use:
   The Varelisa PR3 ANCA EIA kit is used to determine proteinase 3 anti - neutrophil cytoplasmic antibodies in serum or plasma to aid in the diagnosis of Wegener's granulomatosis.

3. Special condition for use statement(s):
   For prescription use only

4. Special instrument Requirements:
   Microplate reader capable of measuring OD at 450 nm

I. Device Description:

The device is an enzyme-linked immunosorbent assay (ELISA) using microtiter plates as the solid phase. The plate wells are coated with PR3 antigens, which allow anti-PR3 antibodies to react with the immobilized antigen (sample). The conjugate is anti-human IgG horseradish peroxidase (HRP), which uses 3, 3'5, 5' tetramethylbenzidine dihydrochloride (TMB) as substrate. The kit contains 6 concentrations of calibrators and controls.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   INOVA Quanta Lite PR3
2. Predicate K number(s):
   k981328
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Indications for Use	To aid in the diagnosis of Wegener's granulomatosis	To aid in the diagnosis of Wegener's granulomatosis
Antigen	PR3 antigen	Same
Substrate	TMB	Same
Assay principle	Indirect noncompetitive enzyme immunoassay	Same
Sample dilution	1:101	Same
Differences		
Item	**Device**	**Predicate**
Specimen matrix	Serum and plasma	Serum
Conjugate	IgG HRP (Anti-human)	Anti-human IgG
Calibrators	6 prediluted calibrators	Prediluted high and low positive
Result interpretation	Semi-quantitative Negative <6 U/mL Equivocal 6-9 U/mL Positive >9 U/mL Qualitative (ratio) Negative = <1.0 Equivocal = 1.0 – 1.4 Positive = > 1.4	Sem-quantitative Negative <20U/mL Weak positive 21-30U/mL Moderate/strong positive >30 U/mL Qualitative (ratio) Negative <1.0 Weak positive 1.0-1.5 Moderate positive 1.6-2.5 Strong positive >2.5

K. Standard/Guidance Document Referenced (if applicable):
None referenced.

L. Test Principle:
Varelisa PR3 ANCA is an indirect non competitive enzyme immunoassay. The wells of the microplate are coated with human PR3 antigen. Antibodies specific for PR3 present in the sample bind to the antigen. The conjugate is anti-human IgG Horseradish peroxidae (HRP) and the substrate tetramethylbenzidine (MB) is added and incubated to develop color. The rate of color formation (measured at OD 450 nm) is proportional to the initial concentration of the respective antibodies in the patient sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:
The purpose of the precision study was to investigate variation within and between assays. The samples (low, medium, and high) were used in a standard 1:101 dilution and were analyzed in 5 runs, with 16 replicates per run. Calibrator and controls were analyzed in duplicates. One operator carried out the analyses within one day. The target values set for the studies were: within and between run variance should be <10% and <15% respectively. The within run % CV ranged from 4.8%-5.9% and between run ranged from 2.4%-9.3%.

b. Linearity/assay reportable range:
Dilution linearity:
The purpose of the dilution linearity study was to demonstrate linearity of the assay over the measuring range. Beginning with a standard dilution of 1:101, the samples were further diluted 1/1, 2/3, 1/4 – 1/32 using sample diluent. Calibrators, controls and each dilution step were analyzed in duplicate. The optical density was measured in a SLT Spectra II at 450nm with a reference filter at 620.

Specifications set for the study were: the observed/expected values should be within ±20% for at least 3 successive dilution steps of each tested sample. Data for all tested samples (6) met the specifications.

Recovery:
The purpose of this study was to demonstrate that the assay detects added amounts of antibodies. Two samples were used in a 1/101 dilution. The samples were spiked with 1/10 volume of calibrator points S1, S2, S3, S4, S5 and S6 (900µL sample plus 100µL calibrator). The spiked samples were analyzed in duplicates. The OD was measured at 450 nm with a reference filter at 620 nm. The

% recovery of all tested samples was with in ±20% of the expected values.

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c. *Traceability (controls, calibrators, or method):*
There is no recognized reference material for PR3 antibodies. Results are reported in arbitrary units

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d. *Detection limit:*
The analytical sensitivity study was performed to verify the detection limit and to show the ability of the assay to differentiate between the background and the first calibrator point. The sample diluent was diluted according to the directions for use and measured 56 times on one plate. Calibrators and controls were analyzed in 4 replicates.

Analytical sensitivity was calculated as mean of optical density (OD) of the sample diluent plus 3 times Standard Deviation (SD), expressed in U/mL. Analytical sensitivity for sample diluent was <0.1 U/ml, calibrator 2 (3 U/mL) %CV was 6.7, discrimination value (D) = 13.4. Calibrator 2/2 (1.5 U/mL) % CV was 1.9 and D = 18.8. Calibrator 2/4 (0.75U/mL) %CV was 5.8 and D = 8.3. The (mean + 3SD) of the OD of the sample diluent was lower than calibrator point S2, lower than 1.0 U/mL and D was above 2.0. Thus specifications for the study were met. Analytical sensitivity of the assay is stated as 0.5 U/mL and measuring range as 0.5 U/mL to 100 U/mL.

e. *Analytical specificity:*
The purpose of the study was to investigate whether high concentrations of potentially interfering substances in serum including bilirubin, hemoglobin, chyle, and rheumatoid factor (RF) adversely affect results of the assay. Three serum samples were diluted 1:101 in sample diluent and spiked with different amounts of interfering substances or their respective blank solutions and analyzed in triplicate. The calibrators and controls were run in duplicate. Specification to be met for this study was: the deviation of the values of the sample spiked with the interfering substance should be less than ±20% of the value of the sample spiked with a buffer blank. The spiking of high concentrations of the potentially interfering substances showed no significant influence on the test results. The specification for this study was met.

f. *Assay cut-off:*
A study was performed to establish and confirm the defined cut-off by measuring 432 apparently healthy blood donor samples, equally distributed by gender and age. Specifications for the study were: 95% of the normal population should be negative. Therefore the 95th percentile should lie below the lower limit of the equivocal range.

The cut-offs were set as <15 U/mL is negative, 15-30 U/mL is equivocal and >30 U/mL is considered positive. The 95th percentile was 1.26 U/mL.

2. Comparison studies:
   a. *Method comparison with predicate device:*
   The comparison was made by testing 270 clinically defined sera (15 Churg –Strauss-Syndrome, 50 microscopic polyangitis, 20 non ANCA associated vasculitides, 16 necrotizing crescentic glomerulonephritis, 40 rheumatoid arthritis, 102 Wegener's granulomatosis, 10 Morbus Crohn, 10 ulcerative colitis, 7 other diseases). These samples were collected from 2 European labs. The patient samples were diluted 1:101 in the sample diluent. Samples, Controls and Calibrators were analyzed in duplicates. Six-field analysis showed a positive agreement of 85.1% (63/74), a negative agreement of 93.4% (183/196) and an overall agreement of 91.1% (246/270). Line data is available in the submission.

   b. *Matrix comparison:*
   The predicate device uses serum only. The new device recommends use of both serum and plasma. A study was performed to demonstrate that the new assay gives the same results for serum, heparin plasma, citrate plasma and EDTA plasma collected from the same specimen. 10 PR3 antibody negative and 10 anitibody positive samples collected from serum bank at Pharmacia Deutschland GmbH at Freiburg. Negative samples were spiked with 10 positive sera and were run in 4 replicates. Calibrators and controls were run in duplicates. Specifications were –difference between serum and plasma for positive samples should not be higher than ±20% for positive samples. Negative samples should be negative as serum or plasma sample. Test results showed (line data available in the submission) no difference higher than ±20%.

3. Clinical studies:
   a. *Clinical sensitivity:*
   Not provided

   b. *Clinical specificity:*
   Not provided

   c. *Other clinical supportive data (when a and b are not applicable):*
   Not applicable

4. Clinical cut-off:
   Not provided

3. Expected values/Reference range:
   Expected value in normal population is negative. Healthy asymptomatic individuals may test positive for PR3 ANCA. Expected values may vary depending on the population tested. Data provided showed the frequency distribution in the 432 healthy subjects as: mean =0.7U/mL, mean+2SD = 2.1U.mL, median =0.6U/mL, 95% percentile =1.2U/mL. The subjects were Caucasian individuals equally distributed by sex and age.

N. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.